ANTI HEMOPHILIC GLOBULIN ACTIVITY OF STORED HUMAN PLASMA

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by

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DEDICATION

To my parents

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INTRODUCTION

Ever since Lane (1) first successfully treated a case of hemophilia in 1840 by means of a blood transfusion, this has gradually come to be the treatment of choice for handling bleeding crises in hemophiliac patients.

At the present time hemophiliac patients at the time of a bleeding crisis are either given fresh plasms or fresh frozen plasms because it has been felt that plasma stored in some other way rapidly loses its AHG* activity (2-6). However, with respect to this there has, in recent years, evolved quite a controversy among different investigators. One group of investigators (7-13)in the past few years have reported that AHG activity is more stable under the usual storage methods of blood bank blood than was formerly thought. If this is the case, it would be of great value in the treatment of hemophiliac patients since it would both insure a readily accessable supply of AHG and would substantially reduce the cost involved in maintaining adequate supplies of fresh frozen plasma. If AHG activity is stable under normal storage conditions, one could effectively use plasma for transfusing hemophiliac patients that is often discarded when packed cells are used for blood transfusions

* Anti hemophilic globulin.

or plasma that is discarded after blood has been stored for more than three weeks. Also it would make available a source of AHG at all hospitals and would eliminate the need of a central clearing agency that is now required for the preparation and distribution of fresh frozen plasma. Therefore, it was felt that the importance of studying the storage stability of AHG activity was a very pressing issue, and for this reason, this study was undertaken.

Much of the controversy over the storage stability of AHG activity has been caused by the fact that until recent years, oxalate instead of an acid citrate dextrose solution, has been used as a blood anticoagulant. Spaet (8) has shown that oxalated blood rapidly loses its AHG activity in sharp contrast to blood stored with ACD, which retains AHG activity for a considerable length of time when stored at 4^{o} centigrade.

Other factors in the collection and storage of blood besides the type of anticoagulant used have been found to play a part in the amount of AHG activity preserved. These have included the type of container used (10) and the care in the collection of the blood (13).

Another factor that has added to the confusion in this matter of AHG activity of stored blood has been the wide diversity of methods used to investigate the amount of AHG activity present in a particular blood sample. These methods

have included clotting time, prethrombin consumption time and the thromboplastin generation test.

Because of these many varied factors the picture regarding the storage stability of AHG activity has become greatly confused. This present study was an attempt to clarify the picture somewhat by acertaining the storage stability of AHG activity of blood collected under various conditions and stored at 4° centigrade.

In the belief that small clots occurring during the collection of the blood might play a part in the storage stability of AHG activity, half of the blood samples were collected after the donar had received intravenous heparin.

Also included is a study of AHG activity of random blood bank samples and an attempt to correlate these findings with those of the study mentioned above.

METHODS AND MATERIALS

Blood was collected from four subjects with known normal prothrombin consumption by means of a standard polyethylene blood donor tube with the blood being collected in quantities of 20 ml. and placed in appropriate storage containers under sterile conditions. It was then stored for varying lengths of time and then AHG assays were done according to a slight modification of the method of Spaet (14).

Collection and Storage of Blood.

Series I. Blood was collected from a normal donor by means of a number 16 needle and polyethylene tubing directly into ten 20 ml. sterile glass test tubes in which a vacuum had been created. Half of these tubes contained 3 ml. of 0.2M ACD* and the other half 2 ml. of 1.0 per cent versene. Four of the samples, two with ACD and two with versene, were manually agitated for five minutes. Two of these agitated samples, one with ACD and one with versene were then centrifuged for five minutes at 2000 revolutions per minute and the plasma removed and placed in sterile glass test tubes. The four samples were then stored at 4° centigrade. Four more of the original samples, two with ACD and two with

* Acid citrate dextrose solutions used for these studies contained: 2.45 grams dextrose; 0.8 grams citric acid; 22 grams orisodium citrate per 100

versene, were centrifuged for five minutes at 2000 revolutions per minute and the plasma removed and placed in sterile test tubes. Two of these samples, one with ACD and one with versene, were stored at 4° centigrade and the other two were frozen. The remaining two original samples were stored at 4° centigrade.

<u>Series II.</u> Blood was collected from the same donor and treated in the same way as in Series I except that the donor was given 10 mg. of heparin intravenously five minutes before the blood was collected.

<u>Series III.</u> The same procedure as for Series I was used except that a different donor was used.

<u>Series IV.</u> The same procedure as for Series I was used except that a different donor was used.

<u>Series V.</u> The same procedure as for Series I was used except for the following changes:

1. Blood samples were collected in siliconized tubes.
2. Samples receiving agitation were not included in this series.
3. A different donor was used.

<u>Series VI.</u> The same procedure as for Series II was used except for the changes noted in Series V.

Series VII. The same procedure as for series I was used except for the following changes:

1. Blood samples were collected in plastic bags.
2. Samples receiving agitation were not included in this series.
3. A different donor was used.

Series VIII. The same procedure as for Series II was used except for the changes noted in Series VII.

AHG Assay Technique

After the blood samples had been stored for three weeks AHG assays were done as follows:

Five one hundreds ml. of the test plasma was added to 2.5 ml. of freshly drawn blood of a known hemophiliac patient whose prothrombin consumption was known to be stable at a level of 65 per cent for a period of six months. The blood was then allowed to clot and was placed in a water bath at a temperature of 37° centigrade for four hours. The serum was then separated by centrifuging and was added to 0.2M sodium citrate so that the final solution contained one part sodium citrate to nine parts serum. This was done to prevent further utilization of prothrombin. This solution was then incubated in a water bath at a temperature

of 37° centigrade for thirty minutes to destroy the excess thrombin and was then subjected to prothrombin determinations according to the method of Stefanini and Crosely (15).

The above concentration of test plasma was used because of reports by Spaet (14) and Mersky (16) that concentrations of one to two per cent fresh frozen citrated plasma are needed in order to make the prothrombin consumption test of hemophiliac patients normal.

Determinations for AHG activity by this means were made on the samples at different time intervals ranging from three to six weeks.

AHG Assay of Stored Blood Bank Blood

Random samples of plasma were taken from stored whole Blood Bank blood that had been collected at Hartford and New Haven and had been stored for periods ranging from three to six weeks and AHG assays were done as described above. All the blood was stored in glass containers and contained ACD as an anticoagulant.

Conversion of Prothrombin Consumption to AHG Activity

Fresh citrated plasma was diluted with saline in different concentrations so that when it was added to 2.5 ml.

of blood from the hemophiliac patient, the final concentration of fresh plasma was 0 per cent, 0.5 per cent, 1.0 per cent, 1.5 per cent, 1.75 per cent, and 2 per cent. Prothrombin consumption tests were then done as described above and the results plotted in Figure 1 (page 20). The abscissa was defined as AHG activity with 0.0 per cent concentration of test plasma being taken as 0 AHG activity and 2 per cent concentration of test plasma as 100 per cent AHG activity. From this graph the prothrombin consumptions determined above were converted to AHG activity.

RESULTS

As can be seen from Tables 1 to 8 and Figure 2, AHG activity as measured above appeared to be well preserved at 4° centigrade when ACD was used as an anticoagulant but was very unstable when versene was used as the anticoagulant. Factors such as type of container, whether the blood was agitated before storage and whether plasma was separated from red cells appeared to have no appreciable effect on the storage stability of AHG activity at least for the first three weeks. Also whether the donor was given heparin before the blood was collected did not appear to influence the results one way or the other.

After three weeks all samples stored in ACD showed AHG activity above 75 per cent where as those stored in versene at 4° centigrade showed AHG activity of 0 per cent.

In all the tested samples, fresh frozen plasma seemed to offer little, if any, advantage over plasma stored at 4° centigrade except where versene was used as an anticoagulant and here fresh frozen plasma had a slight AHG activity after three weeks.

As can be seen from Table 9 and Figure 3, AHG activity of stored Blood Bank blood as measured by the above method varied quite randomly and seemed to be completely

independent of where the blood was collected and how long it had been stored. AHG activity ranged from 25 per cent to 90 per cent.

TABLE 1

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks of Storage	ACD as Anticoagulant					Versene as Anticoagulant				
	Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma	Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma
3	88%	85%	85%	88%	85%	0%	0%	0%	0%	35%
4	88%	75%	80%	80%						
5	88%	75%	75%	75%						
6	80%	75%	50%	70%						
7					70%					0%

NOTE: Blood stored in glass test tubes.

TABLE II

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks of Storage	ACD as Anticoagulant: Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma	Versene as Anticoagulant: Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma
3	85%	88%	88%	88%	88%	0%	0%	0%	0%	45%
4	80%	80%	85%	80%						
5	75%	75%	85%	70%						
6	75%	70%	75%	45%						
7					75%					0%

NOTE: Blood stored in glass test tubes. Donor also received 10 mg. of intravenous heparin five minutes before donating blood.

TABLE III

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks of Storage	ACD as Anticoagulant					Versene as Anticoagulant				
	Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma	Plasma	Whole Blood	Plasma + Agitation	Whole Blood + Agitation	Frozen Plasma
3	80%	85%	80%	75%	88%	0%	0%	0%	0%	30%
4	70%	70%	65%	65%						
5	65%	60%	60%	65%						
6	30%	20%	30%	10%						
7					65%					0%

NOTE: Blood stored in glass test tubes.

TABLE IV

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks of Storage	ACD as Anticoagulant: Plasma	ACD as Anticoagulant: Whole Blood	ACD as Anticoagulant: Plasma + Agitation	ACD as Anticoagulant: Whole Blood + Agitation	ACD as Anticoagulant: Frozen Plasma	Versene as Anticoagulant: Plasma	Versene as Anticoagulant: Whole Blood	Versene as Anticoagulant: Plasma + Agitation	Versene as Anticoagulant: Whole Blood + Agitation	Versene as Anticoagulant: Frozen Plasma
3	80%	85%	80%	80%	85%	0%	0%	0%	0%	10%
4	70%	70%	40%	80%						
5	65%	40%	40%	60%						
6	30%	20%	10%	30%						
7					40%					0%

NOTE: Blood stored in glass test tubes
Donor also received 10 mg. of intravenous
heparin five minutes before donating blood.

TABLE V

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks Stored	ACD as Anticoagulant			Versene as Anticoagulant		
	Plasma	Whole Blood	Frozen Plasma	Plasma	Whole Blood	Frozen Plasma
3	88%	88%		45%	40%	
7	80%	80%	85%	0%	0%	0%

NOTE: Blood stored in siliconized test tubes.

TABLE VI

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks Stored	ACD as Anticoagulant			Versene as Anticoagulant		
	Plasma	Whole Blood	Frozen Plasma	Plasma	Whole Blood	Frozen Plasma
3	90%	75%		10%	45%	
7	80%	70%	80%	0%	0%	10%

NOTE: Blood stored in siliconized test tube. Donor also received 10 mg. of intravenous heparin five minutes before donating blood.

TABLE VII

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks Stored	ACD as Anticoagulant			Versene as Anticoagulant		
	Plasma	Whole Blood	Plasma	Plasma	Whole Blood	Frozen Plasma
3	85%	90%		40%	10%	
7	80%	85%	85%	0%	0%	0%

NOTE: Blood stored in plastic bags.

TABLE VIII

PER CENT AHG ACTIVITY OF STORED PLASMA

No. of Weeks Stored	ACD as Anticoagulant			Versene as Anticoagulant		
	Plasma	Whole Blood	Plasma	Plasma	Whole Blood	Frozen Plasma
3	85%	90%		45%	35%	
7	80%	88%	80%	0%	0%	10%

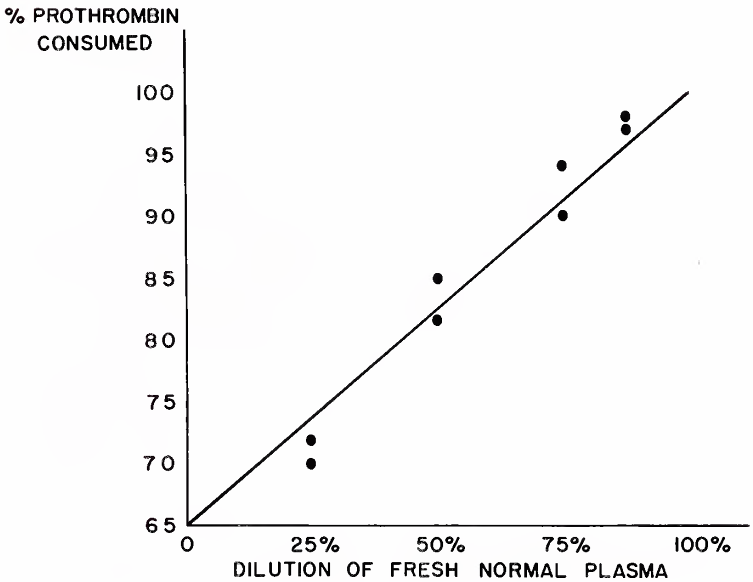
NOTE: Blood stored in plastic bags. Donor also received 10 mg. of intravenous heparin five minutes before donating blood.

TABLE IX

AHG ACTIVITY OF STORED BANK BLOOD

	Length of Storage (weeks)	Where Collected	Activity
1	4	Hartford	30%
2	4	Hartford	60%
3	5	Hartford	40%
4	4	Hartford	70%
5	4	Hartford	85%
6	5	Hartford	35%
7	5	Hartford	75%
8	3	New Haven	75%
9	4	New Haven	30%
10	4	New Haven	25%
11	3	New Haven	65%
12	6	New Haven	90%
13	5	New Haven	75%
14	5	New Haven	30%
15	4	New Haven	85%
16	6	New Haven	70%

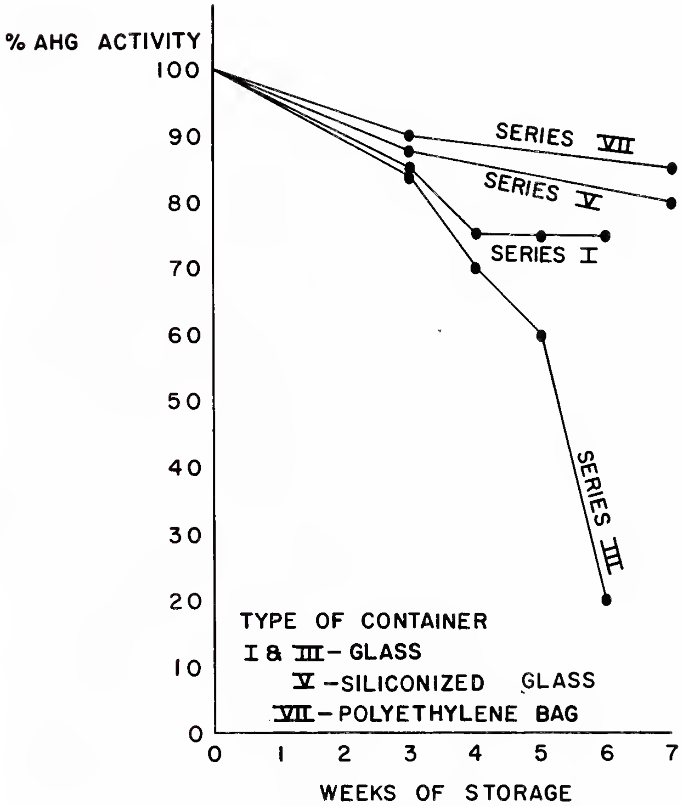
CONVERSION OF PROTHROMBIN CONSUMPTION TO AHG ACTIVITY



GRAPH REPRESENTS A STANDARD DILUTION CURVE FOR % OF NORMAL AHG ACTIVITY AS REPRESENTED BY PROTHROMBIN CONSUMPTION WHEN MIXED WITH THE BLOOD OF A HEMOPHILIAC PATIENT IN A RATIO OF 1 TO 50

FIGURE 1

EFFECT OF TYPE OF STORAGE CONTAINER ON AHG ACTIVITY



EACH LINE REPRESENTS WHOLE BLOOD OBTAINED FROM SEPARATE DONORS AND STORED AT 4° C

FIGURE 2

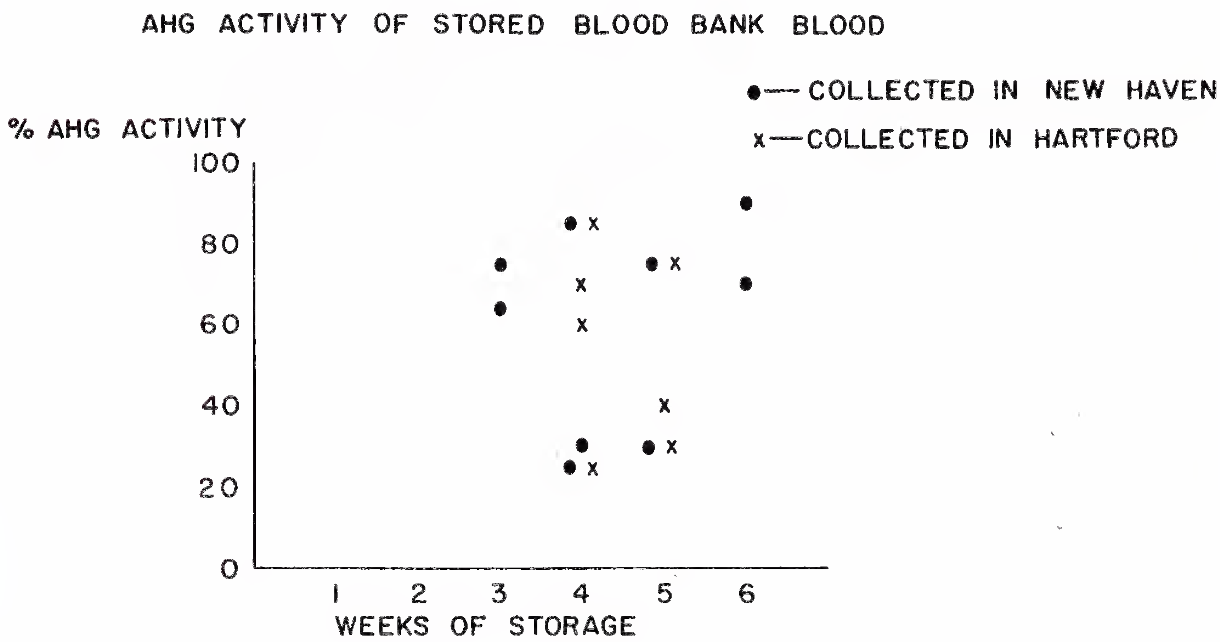

AHG ACTIVITY OF STORED BLOOD BANK BLOOD
●— COLLECTED IN NEW HAVEN
x— COLLECTED IN HARTFORD
% AHG ACTIVITY
100
80
60
40
20
0
1
2
3
4
5
6
WEEKS OF STORAGE

FIGURE 3

DISCUSSION

In the past few years, a host of agents has been investigated both for the management of bleeding emergencies and for prophylaxis against such episodes in hemophiliac patients in an attempt to find a readily available agent and one that would not be too expensive. These have included estrogens (17,18), Hydergine (19,20) (a mixture of ergot alpaliods), intravenous histamine (21) oral vitamin E (22), oral thrombin (23) intravenous organic di iodo compounds (24), intravenous calcium (25), oral glycocoll combined with ascorbic acid and calcium (26), intravenous polyvinylpryrrolidone (27), oral cortisone (28), a concentrate made from human urine (29) and bovine AHG (30). However, there has been no confirming support for any of these agents and there has been no real rationale for their use. Therefore, one is forced to the conclusion that the only available agents effective in the management of hemophiliac patients are human blood and its derivatives.

The results of this series of experiments show that there is a definite possibility that hemophiliac patients can be treated successfully with stored plasma since AHG activity seems to be fairly well preserved if the blood is collected very carefully and stored with ACD as an anticoagulant. This would mean that there could be readily available and at little

cost, a constant supply of plasma for the treatment of hemophilia.

From the work done in this project and from the work of others, it would appear that the most important factors involved in preserving the AHG activity of blood when stored at 4° centigrade are the type of anticoagulant used and the care used in collecting the blood.

With respect to the type of anticoagulant, it has been shown by Spaet (2,8) that all AHG activity is lost within one week when blood is stored in oxalate. Since oxalate was routinely used up until recent years as the anti-coagulant for Blood Bank blood it is easy to see why stored plasma came into such disrepute as a means for treating hemophiliac patients. The results of this experiment also show that AHG activity is rapidily lost when blood is stored in versene. ACD on the other hand does not seem to interfere at all, with the AHG activity of blood or plasma stored at 4° centigrade.

The other important factor in preserving AHG activity seems to be the care with which the blood is collected. Brinkhaus (12,13) has shown that if blood is very carefully collected and preserved in ACD, the AHG activity is well maintained for periods up to three weeks when stored at 4° centigrade. The fact that AHG activity is

well maintained in carefully collected blood may well be related to the fact that if care is taken in collecting the blood, small clots are not allowed to form. It was with this theory in mind that intravenous heparin was given to the donor before half of the samples were collected. However, the fact that heparin did not appear to effect the results of AHG activity is understandable when it is realized that under the conditions of collecting the blood, there was little chance for clotting to occur. The other evidence obtained from this series of experiments, however, does add some weight to this theory. Since the blood was collected fairly rapidly and in small quantities there was very little opportunity for clotting to occur and there was very little loss of AHG activity in any of the samples stored in ACD over a period ranging from three to six weeks. On the other hand the stored Blood Bank blood which was collected routinely showed great variation with respect to AHG activity and it is a known fact that stored Bank blood often contains small clots of blood. Spaet (8)also found this variability in AHG activity of Blood Bank blood but he attributed this variation to an AHG inhibitor that was present in certain normal individuals and that led to the fairly rapid loss of AHG activity. Since the first part of this experiment only used four different donors, one cannot be sure that Spaet's postulated AHG

inhibitor does not exist. To show that the care in preventing any clotting to occur rather than the presence of an AHG inhibitor was responsible for the loss of AHG acitivity of Bank blood would require one to use a large number of donors. The blood would be collected as was done with the four donors in this experiment and then tested to see if AHG activity was retained uniformly all the blood samples or whether it showed a random variation as did stored Bank blood. Were this latter result the case, one would have to give further thought to the presence of an AHG inhibitor as postulated by Spaet.

Another factor that has received some study as exerting influence on the AHG activity of blood stored at 4° centigrade has been the type of container used. Penick and Brinkhaus (10) reported that AHG activity was inhanced by the use of containers with silicone surfaces. The results from the present experiments show that the type of storage container has little effect on AHG activity for the first three weeks of storage. However, for longer periods of time blood and plasma stored in plastic bags or siliconized test tubes seemed to retain AHG activity better than blood stored in glass containers.

Other observations worthy of note are the effect of agitation and the effect of red blood cells on AHG

activity. Agitation did not appear to be detrimental at all, nor did the presence of red cells in spite of their known thromboplastic activity.

From all that has been said, one cannot help but be optomistic with respect to the finding of a readily available agent that can be used for the treatment of hemophilia. It would seem that fresh frozen plasma has nothing more to offer with respect to AHG activity than does blood stored under normal Blood Bank conditions if it is carefully collected and stored in ACD. If it should be found that if clotting occurring during the routine collection is the cause for the random low AHG values in stored blood, it is not inconceivable that blood donors, before donating, could receive intravenous heparin in order to insure a minimum of clotting. Since heparin was shown not to decrease AHG activity this could have no adverse effect on the storage stability of AHG activity. Thus hemophiliac patients, no matter where they were located, would have at the nearest hospital an available and relatively inexpensive source of plasma to combat any crises that might arise.

SUMMARY

1. Plasma AHG activity of blood carefully collected in ACD solution and stored at 4° centigrade is well preserved for periods up to six weeks or longer.

2. Non wetting surfaces appear to be superior to glass surfaces in the preservation of AHG activity of plasma stored at 4° centigrade for six weeks or longer.

3. The AHG activity of plasma obtained from blood collected in versene and stored at 4° centigrade is destroyed rapidly.

4. The sporadic loss of plasma AHG activity in blood collected for the Blood Bank and stored at 4° centigrade probably is due to partial blood clotting rather than the presence of an AHG inhibitor.

5. The plasma AHG activity of stored blood is not effected by agitation, the presence of red blood cells or the presence of small amounts of heparin.

6. The plasma from blood routinely collected into plastic bags containing ACD and stored at 4° centigrade for periods up to seven weeks should be as effective as fresh frozen plasma of comparable age in the treatment of bleeding hemophiliac patients.

BIBLIOGRAPHY

1. Lane, S.: Hemorrhagic diathesis; successful transfusion of blood. Lancet, 2:185, 1840.

2. Spaet, T. H.; Kinsell, B.G.: Properties of bovine anti-hemophilic factor. Proc. Soc. Exp. Biol. Med., 84:314-317 (Nov.) 1953.

3. Howell, W.H.: Hemophilia, Bull. N.Y. Acad. Med., 15:3-26, (Jan.) 1939.

4. Biggs, R.; MacFarlane, R.G.: Human blood coagulation and its disorders. Springfield, Illinois; Charles and Thomas, 1953.

5. Shinowara, G.Y.: Stability of thromboplastic plasma component in human blood and its derivatives. Fed. Proc., 13, 296, (Mar.), 1954.

6. Pitney, W.R.; Dacie, J.V.: Generation of thrombin in recalcified plasma. J. Clin. Patho., 6:9-14 (Feb.) 1953.

7. Taylor, F.; Zogner, E.; Davidson, C.; Tagnon, H.; Newhouser, L.: Preservation of normal human plasma in the liquid state. J. Clin. Invest., 23:351-356, (May), 1944.

8. Spaet, T.H.; Garner, E.S.: Studies on the storage lability of human AHF. J. Lab. & Clin. Med., 46:111-119 (July), 1955.

9. Brinkhaus, K.M; Penick; G.D., Longdill, R.D.; Wagner, R.H.; Grahm, J.B.: Physiologic basis of transfusion therapy in hemophilia. Arch. Path., 61:6-10 (Jan.) 1956.

10. Penick, G.D.; Brinkhaus, K.M.: Influence of storage conditions on stability of AHF of camine and human blood and plasma. Fed. Proc., 13:440-441 (Mar.) 1954.

11. Alexander, B: Anti hemophilic principle of normal plasma. J. Clin. Invest., 26:1173 (Nov.), 1947

12. Brinkhaus, K.M.: Hemophilia. Bull. of N.Y. Acad. Med. 30:325-342, (May) 1954.

13. Brinkhaus, K.M.: Plasma anti hemophilic factor biological and clinical aspects. Le Sange, 25:738-741 (Sept.), 1954.

14. Spaet, T.H.; Kinsell, B.; Behring, H.M.; Aggeler, P.M.: Atypical blood clotting patterns in hemophilia. Stanford Med. Bull., 11:118-123, (May), 1953.

15. Styanini, M.; Crosley, W.: One stage prothrombin consumption test (modified). Blood, 5:964-972, (Oct.) 1950.

16. Mersky, C.: Consumption of prothrombin during coagulation. J. Clin. Path., 3:130-141 (May) 1950.

17. Coari, L.: Valutazione di alcuni estrogeni sui tempo di coagulazione del sangue, sulla trombocitemia e sulla sintomatologia clinica in imofilica. Progr. Med. Nap., 6:727, 1950.

18. Michon, P.; Remigy, E; Vincent, S.: Hemophilies traitees par injections crystallines et implantations d'oestrogenes. Sang., 22:326, 1951.

19. Vodopivec, M.; Jelavic, N.: Wirkung von hydergin auf die gerinnungszeit bei hamaphilie, Aca Haemat., 3:247, 1950.

20. Sturgeon, P.; Friend, W.: The influence of CCK 179 (hydergin) on the coagulation time in hemophiliac children. Acta Haenatol, 6:351-353, 1951.

21. Sanford, H.N.; Bulter, S.; Kennedy, S.R.Sr.: Action of intravenous injection of histamine on the blood of hemophiliac children. Am. J. Dis. Child., 76:609, 1948.

22. Prosperi, P.; Lottini, A.: Le vitimina E, fottore coagulante nella terapia della 'emophilia. Sperimentale, 100:258, 1950.

23. Fiehier, A.: La thrombine per os dons l'hemophilic. Sang. 19:606, 1948.

24. Dunn, D.B.; Lyons, R.N.: The use of organic di iodo compounds in the treatment of hemophilia. M. S. Australia, 2:149, 1951.

25. Kramer, H.; Donovan, W.N.; Beard, M.F.: The favorable response of atypical hemophilia to calcium. U.S.A.F. Med. J., 4:761, 1953.

26. Kohl, H.: Uber eoni oral behandlung von blutungezstanden mit glykokoll askoibin saure kalzium unter bisonderer Berucksichtigung der thrombopenie und der bluterkrankheit. Deutsch. Med. Wschr., 72:279, 1947.

27. Chevallier, S.P.; Guillat, M.; Frehrer, A.: La poly ienylpyrrolidone dons l'hemaphilie. Sang. 20:94, 1949.

28. Luckerini, T.; Natale, P.: Cortisone ed emocoogulazione nella artropatia imoflica. Minerva Med. 43:993, 1952.

29. Tocantins, L.M.; Lindquist, J.N.: Thromboplastic activity of the urine. Proc. Soc. Exper. Biol. Med., 65:44-49, (May) 1947.

30. MacFarlane, R.G.; Biggs, R.; and Bidwell, E.: Bovine AHG in the treatment of hemophilia. Lancet, 266:1316-1319, (June) 1954.

Date Due
